VICH - WG. BIOLOGICALS QUALITY MONITORING.

MINUTES

of the discussion held at NVSL/USDA in Ames, USA, in July 2000.

GUIDELINE FOR THE TESTING FOR EXTRANEOUS AGENTS IN VETERINARY VACCINES.

1. It was decided to divide the guideline for the test for extraneous agents into separate documents which will be approved separately. This decision was taken because it would make work easier and allow earlier approval.

2. It was decided to limit the scope of the guideline to the tests for extraneous viruses in viral veterinary vaccines because not in all regions specific tests for the absence of extraneous agents other than viruses do exist. The limitation of the guideline to viral vaccines was justified because no tests are done for the presence of extraneous viruses in bacterial vaccines.

3. The Guideline for the tests for the presence of extraneous viruses in viral veterinary vaccines will consists of at least the following two documents:

    - Part I. Guideline for the tests for the presence of extraneous viruses in mammalian viral vaccines produced on established cell lines.

    - Part II. Guideline for the tests for the presence of extraneous viruses in avian viral vaccines.

4. The need for a guideline for mammalian viral vaccines produced on primary cells and a guideline for the absence of extraneous viruses in fish vaccines will be considered later.

5. The list of viruses to be tested for shall be reviewed at the next meeting. Specific attention will have to be given to the problems associated with potential contamination with TSE agents. It is expected that additional guidance can be obtained as a result of the specific symposium which will be organised by EDQM on this subject in the beginning of 2001.

6. The draft text of the guideline : "Part I. Guideline for the tests for the presence of extraneous viruses in mammalian viral vaccines produced on established cell lines" was discussed and amended where necessary. A number of questions have still to be resolved. The agreed amended text of the guideline is attached.

7. It was agreed that the guideline shall contain specific sections on tests to be done for intermediate and finished product.

18 September 2000

VICH, International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products.

WORKING GROUP : Biologicals Quality Monitoring.

TOPIC: ***Test on the presence of extraneous agents***

DRAFT TEXT

GUIDELINE

FOR THE TESTS

ON

THE PRESENCE OF EXTRANEOUS VIRUSES

IN VETERINARY VIRAL VACCINES

Date : SEPTEMBER 2000, VS 2000/06

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[4. GUIDELINE FOR TESTING FOR PRESENCE OF EXTRANEOUS VIRUSES IN MAMMALIAN VIRAL VACCINES PRODUCED IN PRIMARY CELLS.

5. GUIDELINE FOR TESTING FOR PRESENCE OF EXTRANEOUS AGENTS IN VACCINES FOR FISH. *(Optional)*]

4. GLOSSARY

5. ANNEXES.

1.INTRODUCTION.

1.1 Objective of the guideline.

It is important that vaccines for veterinary use are free of contaminants, notably viral agents. Potential sources of contamination are the viral strains used for the production of the active ingredient(s), the starting materials of animal origin used in the production of the active ingredient and / or in the assembly of the finished product. Consequently it is necessary to demonstrate that extraneous viruses are not present in veterinary vaccines nor in the starting materials required for their manufacture, through the use of accepted testing procedures and sampling methods and subject to the limitations of the test.

The purpose of the guideline is to provide a description of the test methods to detect the presence of extraneous viruses which shall be undertaken on all materials of animal origin used in the production of veterinary viral vaccines, on intermediate and on the finished product. It shall also provide precise information on the method and conditions of the tests to determine the presence of extraneous viruses in these substances.

1.2. Background.

The materials used in the manufacture of veterinary viral vaccines include:

1. Viral strains
2. Cell substrates
3. Starting materials of animal origin. These materials may be used in the production of the active ingredient and / or in the assembly of the finished product. Strategies to quantify and to minimise the level of risk associated with the use of such materials may include donor herd monitoring, validated treatments, and extraneous agent testing, …..

This guideline deals specifically with the tests to detect the presence of extraneous viruses.

Present methods of testing for extraneous viruses of substances of animal origin are described in the European Pharmacopoeia monograph 62 (1995), the Code of Federal Regulations 9CFR 113 and the OIE Manual of Standards for Diagnostic Tests and Vaccines.

1.3. Scope of the guideline.

The scope of the guideline is to provide guidance on the methods to determine the presence of extraneous viruses and other specified contaminants e.g. Rickettsiae in:

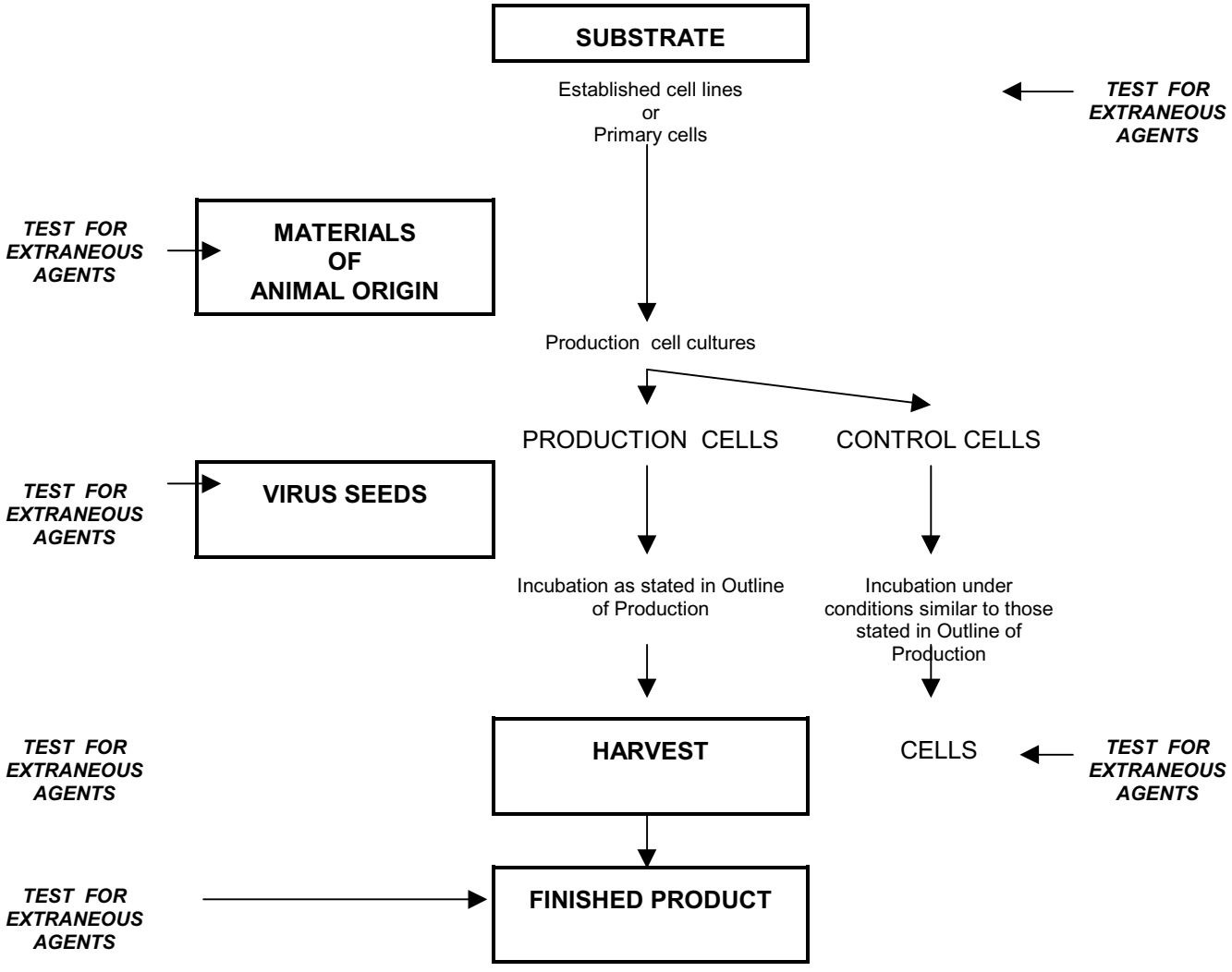
- Master Seed Virus
- Master Cell Seed
- Starting materials of animal origin
- Intermediate products (in-process controls)
- Finished product

SCHEMATIC OUTLINE

OF THE STAGES OF PRODUCTION AT WHICH TESTING OF VETERINARY

VIRAL VACCINES FOR THE PRESENCE OF EXTRANEOUS VIRUSES MAY BE

REQUIRED



2. MAMMALIAN VIRAL VACCINES PRODUCED IN ESTABLISHED CELL-LINES.

CONTENTS.

2.1. TEST FOR THE PRESENCE OF EXTRANEOUS VIRUSES IN VIRUS SEEDS.

2.2. TEST FOR THE PRESENCE OF EXTRANEOUS VIRUSES IN CELL STOCKS.

2.3. TEST FOR THE PRESENCE OF EXTRANEOUS VIRUSES IN MATERIALS OF ANIMAL ORIGIN.

2.4. TEST FOR THE PRESENCE OF EXTRANEOUS VIRUSES IN INTERMEDIATE PRODUCTS (IN-PROCESS CONTROLS)

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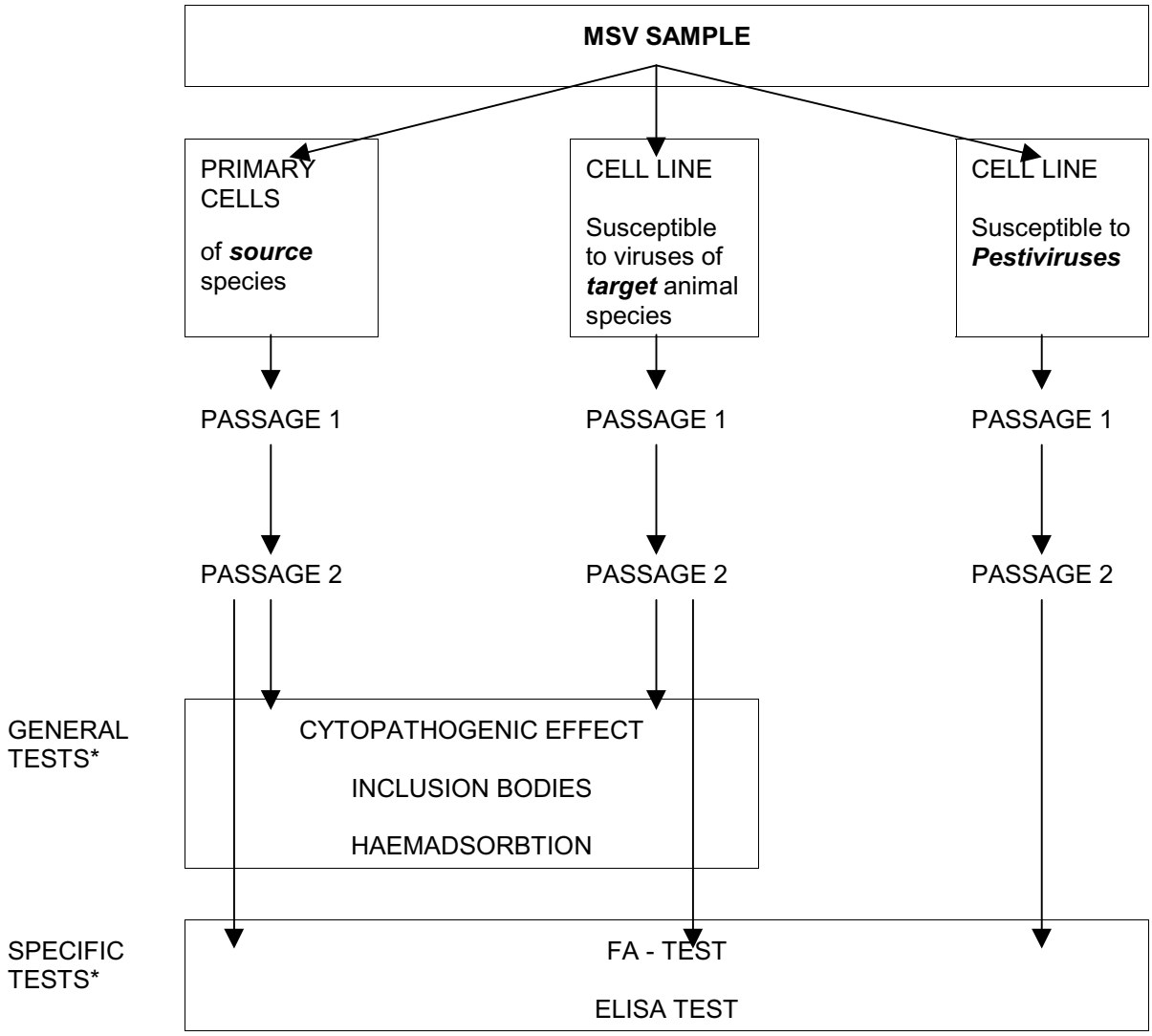
2.1. TEST FOR THE PRESENCE OF EXTRANEOUS VIRUSES IN VIRUS SEEDS

SCHEMATIC OUTLINE OF TEST FOR EXTRANEOUS VIRUSES

MASTER SEED VIRUS



* General tests are tests based on characteristics common to different types of viruses e.g. haemagglutinins while Specific tests are based on characteristics specific to a specific virus or group of viruses e.g. immunofluorescent antibodies.

2.1.1. *General* .

It is required that all viral seed materials are tested to the extent possible for the presence of extraneous viruses. Tests should be carried out for possible contaminating viruses that may originate from:

- the source species of the material.
- from those animal species to which the seed material may have been exposed to e.g. during passaging.

and also

- for the presence of possible contaminating viruses to which the target animal species is susceptible to infection.
-

If during one or more of the preparatory stages of the testing for extraneous agents an additional process is used e.g. adsorbtion it will be necessary to demonstrate that this process does not affect the sensitivity of the test and the testresults.

Seronegative animal inoculation/serology tests may be used specifically for BVD testing.

2.1.2. Samples

NOTE:

The question whether or not tests done on material obtained by not more than 5 passages of the MSV are also valid for the MSV itself, as is allowed by the Eur.Ph., has to be resolved. At this moment USDA requires all tests to be done on the MSV itself.

The Master Seed Virus shall - when necessary - be neutralised with a monoclonal antibody or a polyclonal specific antiserum containing high levels of neutralising antibody to the virus present in the Master Seed Virus.
When a polyclonal specific antiserum is used, it shall be free of antibodies against those viruses for which presence the Master Seed is tested. To that end , such an antiserum will be prepared, wherever possible, with an antigen that

- is not derived from any passage level of the virus isolate giving rise to the Master Seed Virus.
- has been passaged on different cells
- has been adequately purified.

If this is not possible the antiserum may be prepared from an earlier isolate of the virus concerned.

2.1.3. Substrates

The substrates used for testing the presence of extraneous agents shall consist of one or more types of sensitive cells, provided that the three following conditions are met:

- primary cells of the source species;

- cells sensitive to viruses pathogenic for the target species for which the vaccine is intended;
- cells sensitive to pestiviruses. The sensitivity of these cells shall be demonstrated by the use of positive controls e.g. Bovine Viral Diarrhoea virus.

Where relevant, other cells or substrates e.g. eggs, sensitive to other relevant viruses that may potentially be present in the virus seed ,as a result of passaging the virus strain in cells of other animal species shall be used.

2.1.4. Test method

2.1.4.1. Preparation of the substrate cells.

The monolayers of cells of the appropriate sensitivity to be used in the test shall cover an area of at least 75 cm^2 **.**

2.1.4.2. Inoculum.

A quantity of virus, or serum-virus mixture, equivalent to that present in 10 doses of vaccine is inoculated on at least 8 monolayers of suitable cells with a surface of 20 cm^2 each.

Note: The number of monolayers depends on the number of different types of erythrocytes used for haemadsorbtion testing.

NOTE: The size of the sample used for testing has still to be decided upon. It may be necessary to use a type of cascade system or a system based on the MOI used for the production of the Working Seed Virus.

The proposals presented during the meeting in Ames are listed below:

AHI:

Proposal to amend the text:

A 1 mL quantity of virus is inoculated on at least 8 monolayers of suitable cells with a surface of 20 cm^2 [25 cm2 to 150cm2] each.

Reason:

- It should be allowed to inoculate only one monolayer and expand by subculturing at appropriate time intervals to achieve the the number of cultures necessary for the various examinations
- Flexibility is needed on quantity of material when expressed in number of doses. When a killed vaccine is formulated based on ELISA relative potency, it may not be possible to determine how much live virus is present in 10 doses, Also, the final dose of the vaccine may not be known at the time the extraneous agents testing is done.

EU/FEDESA*:*

The quantity of virus shall be related to the quantity of virus used for inoculation as specified in the outline of production.

2.1.4.3. Procedure.

2.1.4.3.1. General tests to detect presence of extraneous viruses.

2.1.4.3.1.1. Observation of cell cultures.

All cell cultures are maintained for at least 21 days, during which at least 2 subcultures are made at 7 days intervals, unless the cells do not survive for this length of time, when the subcultures shall be made on the latest day possible.

They are examined as follows:

- All cultures shall be observed at least every second working day
- At the end of the last subculture

  - 2 monolayers are stained and examined microscopically for cytopathogenic effect, inclusion bodies etc.

  - the other monolayers are examined for haemadsorbtion.

In addition the presence of specific extraneous agents shall be determined by means of immune staining technique using monolayers taken at a time appropriate for the virus under consideration after the initiation of the last subculture, using appropriate controls.

2.1.4.3.1.2. Examination of the cultures for cytopathogenic viruses.

Two monolayers of at least 6 cm^2 each are stained with an appropriate cytological stain. The entire area of each stained monolayer is examined for cytopathogenic effects and any inclusion bodies, abnormal numbers of giant cells or any other lesion indicative of a cellular abnormality which might be attributable to a contaminant.

Precise description of test shall be included in text.

2.1.4.3.1.3. Examination for haemadsorbent viruses.

Monolayers totalling at least 70 cm^2 are washed several times with an appropriate buffer. A sufficient volume of a suspension of appropriate red blood cells, but at least including chicken and guineapig erythrocytes, are added to cover the surface of the monolayer evenly. After incubation for 25-30 minutes at +2 - +8 ° ***C*** ° C and incubation for25-30 minutes at 20-25 ºC and at any other specified temperature and, where necessary under other appropriate conditions, cells are examined for the presence of haemadsorption.

Precise description of test shall be included in text.

NOTE: The question on the number and surface of the monolayers and whether it is allowed to use mixed erythrocyte suspensions has to be resolved.

USDA:

- Number and size of monolayers have to be specified
- Erythrocyte concentration has to be specified. 0.2%
- ***Is is allowed to use mixed erythrocyte suspensions?(different buffers??)***

2.1.4.3.2. Tests to detect presence of specific viruses.

For viruses, which are unlikely to be detected by the general tests described above, specific test methods must be applied. These viruses are indicated under the heading “Specific tests” in the relevant tables.

2.1.4.3.2.1. Preparation of the substrate cells.

The monolayers of cells of the appropriate sensitivity to be used in the test shall cover an area of at least 75 cm^2 **.**

The monolayers are maintained in culture for a total of at least 21 days. At least two subcultures are made at 7-day intervals, unless the cells do not survive for this length of time, when the subcultures are made on the latest day possible.

2.1.4.3.2.4. Examination for specified viruses.

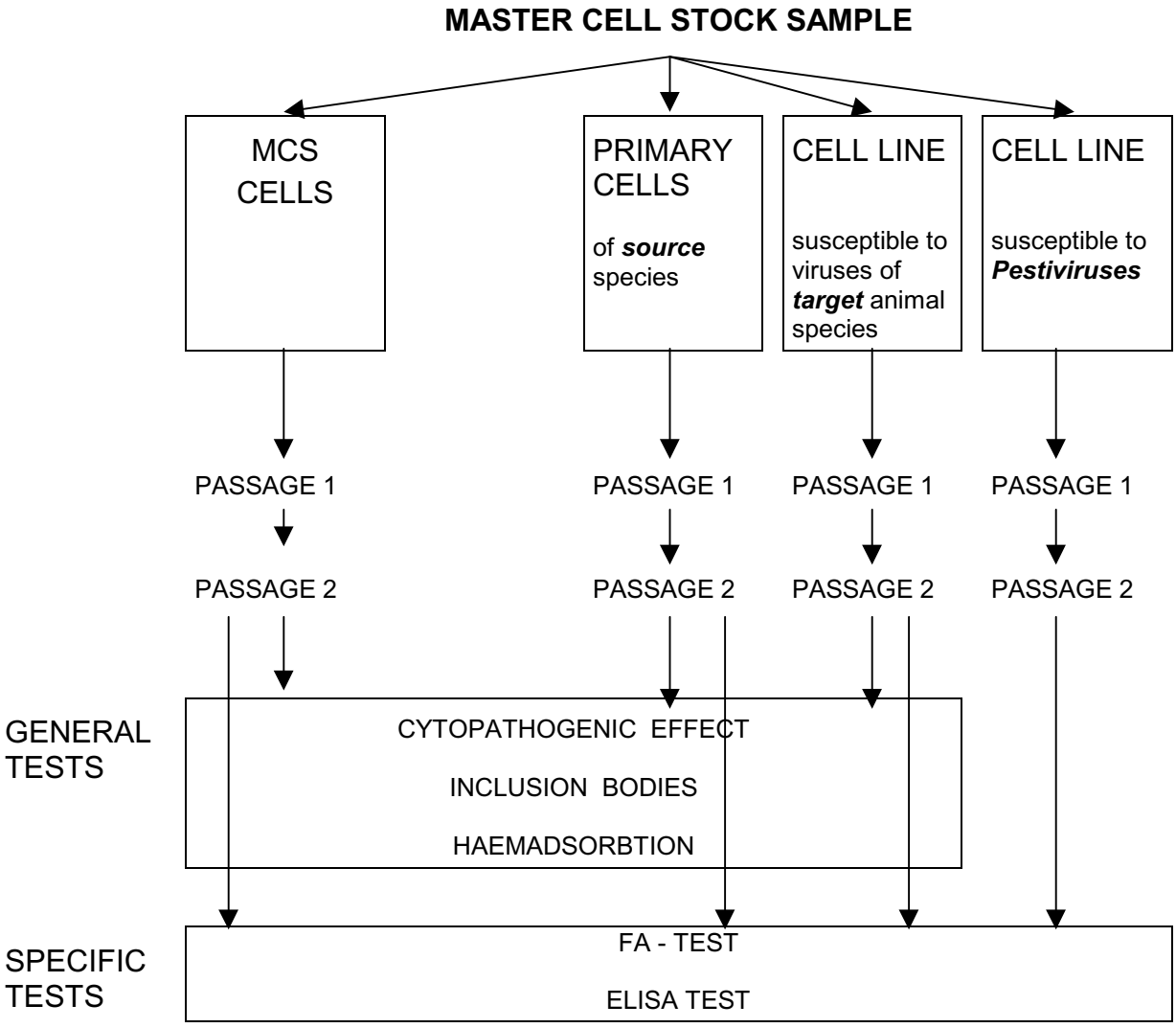
Sufficient cells on suitable supports (e.g. micro-plates or slides) are prepared to carry out tests for the agents specified. The material shall be obtained after at least 2 passages and a total surface area of at least 6 sqcm shall be examined. Suitable positive controls and negative controls are included in each test. The cells are subjected to suitable tests, for example using immune-staining or other techniques.

In specific cases other techniques as e.g. EM and PCR may be considered as well.

2.2. TEST FOR THE PRESENCE OF EXTRANEOUS VIRUSES IN CELL STOCKS

SCHEMATIC OUTLINE OF TEST FOR EXTRANEOUS VIRUSES.

MASTER CELL STOCK.



* General tests are tests based on characteristics common to different types of viruses e.g. haemagglutinins while Specific tests are based on characteristics specific to a specific virus or group of viruses e.g. immunofluorescent antibodies.

2.2.1. General.

It is required that the identity of the cell stock is determined and that the cell stock is tested to the extent possible for the presence of extraneous agents. Tests should be carried out for possible contaminating viruses that may originate from:

- the source species of the material.
- from those species from which materials used in the preparation of the Master Cell Stock has been derived.

and also

- for the presence of possible contaminating viruses to which the target animal species is susceptible to infection.

Seronegative animal inoculation/serology tests may be used specifically for BVD testing.

2.2.2. Samples

The size of the sample shall depend on the number of cells present in the cell seed material but shall contain at least 1 x 10^5 cells per ml. Or sufficient cells to produce a monolayer of at least 75 cm2.
Alternatively monolayers with a total surface of at least 75 cm2 may be used.

The size of the sample taken in the case of suspension cultures shall be at least 1x10^5 cells per ml. or at least 1 ml. of the suspension culture, whichever sample contains the largest number of cells.

2.2.3. Substrates

The substrates used for testing the presence of extraneous agents shall consist of at least one or more different types of sensitive cells provided that the 3 following conditions are met:

- primary cells of the source species, provided they are more sensitive than available non-primary cells ,except in the case of testing well-known established cell lines of simian origin. In the latter case the test may be done on another established cell line of simian origin.

- cells sensitive to viruses pathogenic for the target species cells sensitive to pestiviruses. The sensitivity of these cells shall be demonstrated by the use of positive controls e.g. Bovine Viral Diarrhoea virus.

2.2.4. Test method.

The test to determine the presence of extraneous agents in cells consists of two different stages:

- the test on the initial cell-cultures
- the test on the passaged cell-cultures

2.2.4.1. The test on the initial cell-cultures.

2.2.4.1.1. Preparation of the cell cultures.

The Master Cell Stock is used to establish monolayers in suitable culture vessels.

The monolayers to be used in the test shall cover an area of at least 75 cm^2 and shall be prepared and maintained using medium and additives, and grown under similar conditions – as far as is feasible - to those used for the preparation of the vaccine. The medium used shall be that described in a relevant Outline of Production.

2.2.4.1.2. Procedure.

At least 2 subcultures are made of the monolayers with an interval of 7 days. or as frequently as may be necessary for suitable growth. The cell-cultures are maintained for at least 21 days during which period the cultures are examined regularly for cytopathogenic effect.

At least 7 days after the initiation of the last subculture they are examined :

- for the presence of haemadsorbtion.
- microscopically for cytopathogenic effect, inclusion bodies etc. by staining of 2 monolayers with May Grunwald Giemsa, HE or other suitable staining methods.
- for the presence of specific extraneous agents by means of immuno-staining..

Transmission electronmicroscopy could also be used.

For details see section 2.1.4.3.

2.2.4.2. **The test on the passaged cell-cultures.**

2.2.4.2.1. Preparation of the cell cultures.

The Master Cell Stock is used to establish monolayers with a surface of at least 140 cm^2.

- Note: In the case of non-anchorage cells alternative methods have to be established.

2.2.4.2.2. Procedure.

The cells of the monolayers of each cell type used, are subjected to freezing-thawing (3 times). The material
(1 ml) is then inoculated on each of two monolayers of each of the cell cultures, with a surface of > 75 cm^2,

One subculture is made with an interval of 7 days or as frequently as needed for suitable growth .The cell-cultures are maintained for at least 14 days during which period the cultures are examined regularly for cytopathogenic effect.

At least 7 days after the initiation of the subculture the cells are examined:

- for the presence of haemadsorbtion.
- Microscopically for cytopathogenic effect, inclusion bodies etc. by staining 2 monolayers with May Grunwald Giemsa, HE or other suitable staining methods.
- for the presence of specific extraneous agents by means of immuno-staining.

For details see 2.1.4.3.

2.3. TEST FOR THE PRESENCE OF EXTRANEOUS VIRUSES IN MATERIALS OF ANIMAL ORIGIN.

2.3.1. General .

[*Normally*] the material of animal origin shall be tested before it is subjected to a sterilisation procedure. The material is tested by applying tests of a general nature that may be expected to detect a broad selection of agents, complemented by specific tests to detect individual agents for which there is a particular risk of occurrence. These agents - only of viral nature - are listed in Annex 2.

NOTE: The absence of TSE agents and BVD in serum etc. are very important issues which will have to be resolved. Additional information may be obtained during the proposed EDQM meeting on this subject.

- ***If the material has been heat sterilised testing is not necessary***
- ***Testing shall be done after other sterilisation procedures and not before***.

2.3.2. Samples.

The sample size shall be justified on the basis of likelihood of contamination, degree of purification applied, degree of concentration and availability. In the case of serum 15 % v.v. is recommended.

The material, when prepared for testing, shall be dissolved or suspended in a defined medium, that is identical or at least related to the medium in which the substance will be used in the production process, provided this medium is compatible with the test system.

Any solids are dissolved or suspended in a suitable medium in such a way as to create a solution or suspension containing at least 30 per cent w/v of the substance to be examined.

If the substance is not soluble or where cytotoxic reactions occur, a lower concentration may be used when justified. In case of very scarce ingredients a minimum sample size may be used.

2.3.3. Substrates.

The test shall be done with suitably sensitive cell cultures, including primary cell cultures of the same species of origin as the ingredient to be tested and VERO cells. The monolayers shall have a surface of at least 75 cm^2

Note: Are all known VERO cells equivalent?

2.3.4. Test method.

At least 2 subcultures are made of the monolayers with an interval of 7 days. The cell-cultures are maintained for at least 21 days during which period the cultures are examined regularly for cytopathogenic effect.

At the end of the incubation period the last subculture shall be examined :

- microscopically for cytopathogenic effect, inclusion bodies etc. by staining of 2 monolayers with May Grunwald Giemsa, HE or other suitable staining methods.

- for the presence of specific extraneous viruses

2.3.4.1. Preparation of substrate cells.

The monolayers of cells of the appropriate sensitivity to be used in the test shall cover an area of at least 75 cm^2 .

2.3.4.2. Inoculum.

The size of the sample used for testing has still to be decided upon.

2.3.4.3. Procedure.

At least 2 subcultures are made of the monolayers with an interval of 7 days. or as frequently as may be necessary for suitable growth.The cell-cultures are maintained for at least 21 days during which period the cultures are examined regularly for cytopathogenic effect.

At least 7 days after the initiation of the last subculture they are examined :

- for the presence of haemadsorbtion.
- microscopically for cytopathogenic effect, inclusion bodies etc. by staining of 2 monolayers with May Grunwald Giemsa, HE or other suitable staining methods.
- for the presence of specific extraneous agents by means of immuno-staining.

2.4. TEST FOR THE PRESENCE OF EXTRANEOUS VIRUSES IN INTERMEDIATE PRODUCTS. (IN-PROCESS CONTROLS)

Proposal to be developed by topic leader.

2.5. TEST FOR THE PRESENCE OF EXTRANEOUS VIRUSES IN FINISHED PRODUCT.

Proposal to be developed by topic leader.

GLOSSARY

BATCH (STARTING MATERIAL OF ANIMAL ORIGIN)

The total quantity of the material mixed in a single container and identified by a **unique** serial number.

BATCH (FINAL LOT) VACCINE

A collection of closed, final containers or other final dosage units that are expected to be homogeneous and equivalent with respect to risk of contamination during filling or preparation of the final product. The dosage units are filled, or otherwise prepared, from the same final bulk vaccine, freeze-dried together (if applicable) and closed in one continuous working session. They bear a distinctive number or code identifying the final lot (batch). Where a final bulk vaccine is filled and/or freeze-dried on several separate sessions, there results a related set of final lots (batches) that are usually identified by the use of a common part in the distinctive number or code; these related final lots (batches) are sometimes referred to as sub-batches, sub-lots or filling lots.

CELL-SEED SYSTEM

A system whereby successive final lots (batches) of a product are manufactured by culture in cells derived from the same master cell seed. A number of containers from the master cell seed are used to prepare a working cell seed.

CELL LINES

Cultures of cells that have a high capacity for multiplication *in-vitro.*

NOTE: This point has still to be resolved.

JMAFF Proposal to amend the text as follows:

Cultures of cells that have a high capacity for multiplication in-vitro and can be subcultured continuously.

CELL PASSAGE

The result of an *in-vitro* process whereby cells are replicated, either by being transferred from one container to another or provided with additional growth surface or volume intended to result in renewed or expanded cell growth.

CONTROL CELLS

A quantity of cells set aside, at the time of inoculation, as uninfected cell cultures and which are the same as used in the test. The uninfected cells are incubated under similar conditions to those used for the test

MASTER CELL SEED

A collection of aliquots of cells for use in the preparation of the product, distributed into containers in a single operation, processed together and stored in such a manner as to ensure uniformity and stability and to prevent contamination. Master cell seed is usually stored at temperatures of - 70 °C or lower.

MASTER SEED LOT

A culture of a micro-organisms for use in the preparation of the product, distributed from a single bulk into containers and processed together in a single operation in such a manner as to ensure uniformity and stability and to prevent contamination.

PRIMARY CELL CULTURES

Primary cell cultures are cultures of cells essentially unchanged from those in the animal tissues from which they have been prepared and being no more than 10 *in-vitro* passages to the test level from the initial preparation from the animal tissue. The first *in-vitro* cultivation is regarded as the first passage of the cells.

SEED-LOT SYSTEM:

A seed-lot system is a system according to which successive batches of a product are derived from the same master seed virus. For routine production, a working seed virus may be prepared from the master seed virus.

VIRUS PASSAGE

The result of an *in-vitro* or *in-vivo* process whereby a virus is replicated, either by inoculation of non-infected cells, embryos or animals or performing a cell passage in the case of persistently infected cells.

WORKING CELL SEED

A collection of aliquots of cells derived from the master cell seed and intended for use in the preparation of production cell cultures. The working cell seed is distributed into containers, processed and stored as described for master cell seed.

WORKING SEED VIRUS

A collection of aliquots of a micro-organism derived from the master seed virus and intended for use in production. Working seed virus is distributed into containers and stored as described for master seed virus.